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Effect of Soybean Volatile Compounds on *Aspergillus flavus* Growth and Aflatoxin Production

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ABSTRACT: Soybean homogenates produced volatile compounds upon exposure to lipase. These induced volatiles were identified by SPME. Seventeen volatile compounds identified by SPME were chosen for determination of their ability to inhibit *Aspergillus flavus* growth and aflatoxin B_1 (AFB1) production in a solid media assay. These volatiles included aldehydes, alcohols, ketones, and furans. Of the tested compounds, the aldehydes showed the greatest inhibition of fungal growth and AFB1 production. These compounds inhibited up to 100% of the observed growth and AFB1 production as compared to the controls. The greatest activity by the aldehydes to disrupt growth was ranked as follows: 2,4 hexadienal > benzaldehyde > 2-octenal > (*E*)-2-heptenal > octanal > (*E*)-2-hexenal > nonanal > hexanal. The greatest activity by the aldehydes to reduce AFB1 was ranked as follows: (*E*)-2-hexenal > 2,4 hexadienal > (*E*)-2-heptenal > hexanal > nonanal. (*E*)-2-hexenal and (*E*)-2-heptenal were tested further in an *A. flavus*-inoculated corn kernel assay. Both compounds prevented colonization by *A. flavus* and eliminated AFB1 production when exposed to compound volumes < 10 μL as also shown in the solid media assay. The results suggest that soybeans react to lipase by production of potent antifungal volatiles.

Keywords: aflatoxin, *Aspergillus flavus*, growth inhibition, soybean volatiles

Introduction

Aspergillus flavus is a ubiquitous, saprophytic soil fungus and is considered a weak plant pathogen. Seed coat integrity is the most important barrier against microbial colonization (Stössel 1986; Mellon and Cotty 2002). However, *A. flavus*, *A. parasiticus*, and several other members of the *Aspergillus* genus can gain access to such seeds via damage due to environmental stresses such as drought, excessive heat, or insect damage (Diener and others 1987; Payne 1992). Following the initial infection, these fungi preferentially colonize the lipid-rich embryo and aleurone (Brown and others 1992; Keller and others 1994). Colonization is facilitated by production of enzymes such as pectinase, polygalactronase, and lipase (Cotty and others 1990; Smart and others 1990; Brown and others 1992). During growth, toxic secondary metabolites known as aflatoxins can contaminate economically important oil-rich crops including corn, cotton, peanut, and treenuts (Gourama and Bullerman 1995). Should toxin levels surpass legal tolerance limits, grain can be rendered unsuitable for food or feed use (van Egmond 1991).

Soybean, *Glycine max* (L.) Merr, is another seed rich in oil. Though damaged soybean seed can be colonized by *A. flavus*, it does not generally provide a good substrate for *A. flavus* growth and aflatoxin production (Bean and others 1972; Shotwell and others 1978). Doehlert and others (1993) suggested that the lipoxygenase (LOX) pathway and associated volatiles play a key role in prevention of aflatoxin formation in soybean seed tissues. Later, 13*S*-hydroperoxy fatty acids, metabolites of the LOX pathway were reported to directly or indirectly repress aflatoxin and sterigmatocystin (a toxic precursor of aflatoxin) biosynthesis in *A. flavus* (Burrow and others 1997).

Other research has shown that plant volatiles can inhibit fungal growth and act as an important barrier against colonization (Gueldner and others 1985; Utama and others 2002; Myung and others 2007) and toxin formation (Zeringue and McCormick 1990; Wright and others 2000). Wright and others (2000) examined volatile compounds released from corn for their ability to disrupt growth and aflatoxin production by *Aspergillus parasiticus*. When exposed to 100 μL of one of these volatile compounds, hexanal, radial growth of *A. parasiticus* was retarded and aflatoxin production was reduced. Earlier work in our laboratory showed similar evidence that volatile compounds released by lipase-treated soybean seed homogenate can inhibit both colony growth (dry weight) and aflatoxin produced by *A. flavus* (Boué and others 2005). These volatiles were identified by solid phase microextraction (SPME) combined with gas chromatography–mass spectrometry (GC–MS).

The purpose of the current study was 2-fold. First, a study was performed to determine the ability of induced volatiles from soybeans to inhibit *A. flavus* growth and aflatoxin B1 (AFB1) production on potato dextrose agar (PDA). Seventeen of the identified soybean volatiles were selected for this study. Second, based on the data from the 1st study, the 2 most active volatiles were then tested for their abilities to inhibit *A. flavus* growth and aflatoxin production on corn kernels.

Materials and Methods

Inoculum preparation

Over the course of the experiments, conidial suspensions were prepared and stored at 4 °C for use as inocula. Each suspension was prepared by transferring a 5-mm plug (V8 agar, refrigerated storage in sterile water) of *A. flavus* to potato dextrose agar (PDA) plates

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that were incubated at 30 °C for 6 d. Upon culture maturation, conidia were harvested by flooding the culture dish with 10 mL of 0.5% Tween 20 in water. Prior to each experiment, an aliquot of the stock conidia was diluted to 1×10^6 conidia/mL.

Growth studies

Petri dishes (100 × 15 mm) containing 20 mL potato dextrose agar (PDA) were overlaid with a 90-mm disc cut from a piece of dialysis tubing (Spectra/Por 1, 100 mm flat width, Spectra/Por, Houston, Tex., U.S.A.). Prior to placement on the agar surface, the dialysis tubing disc was placed in water and autoclaved (15 min). After sterilization, the dialysis disc was placed on sterile paper towels to remove excess water. Next, the drained, sterile dialysis disc was placed on the surface of the PDA to create a physical membrane barrier between the *A. flavus* growth and the medium. Prior to each experiment, an aliquot of the stock conidia was diluted to 1×10^6 conidia/mL. An aliquot (250 μL) of the diluted conidial suspension was spread across the surface of each agar plate using a sterile bent glass rod. The inoculated plates were incubated quiescently for 20 to 30 min prior to addition of the volatile samples described below.

Soybean volatile assays

Seventeen induced volatiles (see below) identified earlier (Boué and others 2005) as produced by soybean homogenates in the presence of lipase were tested separately for their ability to inhibit *A. flavus* growth and AFB1 production. These volatiles included benzaldehyde, hexanal**,** nonanal, octanal, (*E*)-2-heptenal, (*E*)-2-hexenal, 2-octenal, 2,4 hexadienal, 1-hexanol, 1-pentanol, 3-octanol, 1-octen-3-ol, 2-heptanone, 3-heptanone, 3-hexanone, 3-octen-2-one, and 2-pentylfuran. All volatiles were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Mo., U.S.A.).

Small caps, removed from 2 mL microfuge tubes (Rainin), were used as reservoirs for each volatile. Treatments consisted of 0, 1, 10, 25, and 50 μL of each test volatile pipetted separately into inverted caps. A single cap was placed in the inverted position on the upper lid of an inverted, inoculated Petri dish. The inverted Petri dishes were sealed with a single layer of parafilm immediately following delivery of the volatile compound to reservoir then incubated at 30 °C for 72 h in the dark. The experimental control dishes (no volatile added) were prepared exactly as those for treatments described previously; however, they did not receive any volatile compound. Each treatment was replicated in triplicate.

A. flavus growth measurement

A 20 × 20 mm piece of membrane with adhering fungal mat was excised from each test sample and control plate at the end of the experiment and transferred to 60 × 15 mm Petri dishes. These dishes were sealed with parafilm prior to microscopy study. The fungal mat present on the surface of the membrane remaining on the agar surface was removed by scraping, transferred to a glass vial and fresh weight was recorded. The orifice of each glass vial was covered with Miracloth (Calbiochem, La Jolla, Calif., U.S.A.), placed in a forced-air oven-dried for 24 h, after which the mycelial dry weight was recorded.

Aflatoxin B1 determination

The respective agar and membrane remaining from the dry weight measurement studies were transferred together to a glass beaker for aflatoxin extraction. A modification of the Zeringue and McCormick (1990) procedure was used to extract AFB1. Agar was minced into very small pieces in a 150-mL glass beaker to which were added 50 mL of 70% acetone/30% water (v/v), then covered with aluminum foil and allowed to stand undisturbed for 30 min at room temperature. Extract was decanted into a separatory funnel to which methylene chloride (50 mL) was added. After the layers were separated, the lower (methylene chloride) layer was passed through sodium sulfate into a 150-mL beaker and allowed to dry passively overnight. The dried extract residue was dissolved in methylene chloride and transferred to a glass vial (7 mL). This solution was dried passively overnight. Dried AFB1 residue was resuspended in 100 μL methanol, then transferred to a 2 mL, 0.45 μ filter centrifuge tube (Spin-X; Corning Inc., Corning, N.Y., U.S.A.) and centrifuged at 14000 rpm for 1 min. The use of methanol was a modification required because the previous researchers used thin layer chromatography whereas the current work employed high-pressure liquid chromatography (HPLC). Preliminary testing showed methanol to be the solvent of choice for the HPLC analyses. After centrifugation the filtered solution was removed. Aliquot samples (10 μL) of the filtered extracts were analyzed by HPLC.

Effect of volatiles on *A. flavus* growth and AFB1 production on corn

The results from the initial experiments indicated that 2 aldehydes, (*E*)-2-hexenal, and (*E*)-2-heptenal, were statistically superior to the other test volatiles in reducing or eliminating *A. flavus* growth and AFB1 production. These compounds were tested further to determine their ability to prevent *A. flavus* growth and AFB1 production on viable corn kernels. Procedures for the assay were, with some modification, performed as described previously (Brown and others 1993). Petri dishes (90 mm dia) were inverted and into each was placed a 90 mm Whatman nr 2 filter paper circle (Whatman, Maidstone, U.K.) moistened with 700 μL of sterile water.

Corn variety Oro 188 was employed in this assay because a previous study (Guo and others 1995) showed that this variety was susceptible to *A. flavus* colonization and was a suitable substrate for AFB1 production. Intact and uniformly sized kernels were selected and surface sterilized in 70% ethanol as previously described (Brown and others 1993). Kernels were inoculated by submergence into *A. flavus* spore suspension (10^6 conidia/mL) for 3 min and used immediately in the experiment.

Subsequently, a smaller Petri dish (60 mm dia) containing 4 plastic caps was placed inside each of the inverted Petri dishes. Into each cap was placed a single, viable corn kernel that had been inoculated with a suspension of *A. flavus* conidia as described previously. After corn kernels were in place, a 5th cap containing the appropriate test volume of either (*E*)-2-hexenal or (*E*)-2-heptenal was also placed in the inverted Petri dish. The inverted Petri dishes were sealed immediately with a single layer of parafilm then incubated at 30 °C for 5 d in darkness. Sample treatments consisted of 4 seeds in a dish. Six dishes per volatile volume were used per run. Three runs were performed. Control treatments were prepared exactly as described previously; however, they did not receive any volume of aldehyde compound.

Determination of *A. flavus* growth on corn kernels

Kernels were observed by microscopy to determine the amount of mycelial growth, conidial development, and color on the kernel surface as well as kernel appearance. *A. flavus* growth on treated kernels was also observed for any morphological differences compared to that of control sets consisting of corn kernels inoculated with *A. flavus* that were not exposed to the respective aldehyde. Corn kernels, including kernels exposed to aldehydes as well as control samples, were dried in a forced-air drying oven at 60 °C. The combined dry weight of all 4 kernels/chamber for the respective aldehyde, per run, was recorded.

Determination of AFB1 in corn kernels

AFB1 was extracted from aldehyde-exposed and control kernels by a procedure similar to that described by Brown and others (1993). All 4 kernels from a treatment dish were combined as a single sample unit for processing and were placed together into a small paper envelope and dried in a forced-air drying oven for 24 h. After drying, corn kernels were crushed with a hammer and the respective dry weight recorded. Each run (3 total) consisting of 24 seeds (4 seeds in each of 6 plates) were processed and analyzed. Crushed samples were transferred to a glass-stoppered flask (50 mL) to which methylene chloride (25 mL) was added. Flasks were agitated 30 min in an orbital shaker. After shaking, the contents of the flasks were filtered into a beaker and allowed to dry passively overnight. Residues were transferred with methylene chloride from beakers to glass vials and allowed to dry passively overnight.

AFB1 sample preparation for HPLC analysis

Each dried sample residue was resuspended in 200 to 300 μL acetonitrile, then transferred to a 2 mL, 0.45 μ nylon filter centrifuge tube (Spin-X; Corning Inc.) and centrifuged at 14000 rpm for 1 min. Aliquot samples (10 μL) of filtered extract were analyzed by HPLC. Acetonitrile was used to extract the dried corn extracts because this solvent is superior to methanol when extracting AFB1 in the presence of corn oil present in the corn kernel extract. The use of acetonitrile produced higher AFB1 recovery and purity than did methanol.

AFB1 analysis was performed similarly to a previously described procedure (Sobolev and Dorner 2002). HPLC analyses were performed with a Waters 2695 HPLC combined with a Waters 2475 fluorescence detector. Postcolumn derivatization was performed with a photochemical reactor for enhanced detection (PHRED, Aura Industries Inc., New York, N.Y., U.S.A.) system. AFB1 detection wavelength was 365 nm (excitation) and 474 nm (emission). Sample extract (10 μL) was injected for separation through a Nova-Pak C18 (3.9 $\times$ 150 mm, 5 μm, Waters Corp., Wilford, Mass., U.S.A.) reverse phase column. The analytical column was protected by a guard column containing the same packing. Column temperature was maintained at 38 °C. Elution flow rate was 0.8 mL/min with mobile phase solvent consisting of water:methanol:n-butanol (1400:720:15, v/v/v). Retention time for AFB1 was 12.3 min. A calibration curve with high linearity ($R^2 = 0.9953$) was constructed for AFB1 from a series of diluted standards. The detection limit for AFB1 using the HPLC protocol was 2.0 ng.

Statistical analysis

Data were analyzed by SAS (1999–2001) general linear models procedure (22). Means were separated using Student–Newman–Keuls test ($P \leq 0.05$).

Results

Inhibition of *A. flavus* growth in assays using PDA as a substrate

Aldehydes. Among all the volatiles tested in this study, the aldehydes influenced the greatest inhibition of growth and AFB1 production (Table 1). *A. flavus* germinated and grew in the presence of each compound at both 0 (control) and 1 μL exposure volume (EV). At 10 μL EV, *A. flavus* colonies formed only in chambers treated with hexanal and nonanal. No colonization was observed in any of the 25 or 50 μL EV treated chambers. Growth was reduced at 1 μL EV by three of the compounds, benzaldehyde (33%), 2,4 hexadienal (56%), and octanal (14%). The remaining 5 aldehydes, hexanal, nonanal, (*E*)-2-heptenal, 2-octenal, and (*E*)-2-hexenal did not influence any growth reduction until 10 μL EV (64, 80, 100, 100, and 100%, respectively).

Alcohols. At 0, 1, and 10 μL EV, *A. flavus* colonized all chambers that received an alcohol compound. The only significant growth reduction detected among those 3 treatments was by 1-octen-3-ol at 1 μL EV (18%) and 10 μL EV (27%). Colony growth was greatly reduced at 25 μL EV in presence of 1-hexanol (74%). However, 1-octen-3-ol and 3-octanol had a germicidal or germistatic effect on the inoculant. At 25 μL EV therefore, no growth occurred in those

Table 1 — *Aspergillus flavus* colony growth and toxin production after 72 h continuous exposure to 0, 1, 10, 25, and 50 μL of isolated soybean compounds.

	Colony dry weight (mg/petri)					Aflatoxin B1 (ng/petri)				
	0	1	10	25	50	0	1	10	25	50
Aldehydes										
Hexanal	130.0 a	113.3 a	47.0 b	0.0 c	0.0 c	1719.3 a	922.4 b	357.1 c	0.0 d	0.0 d
Benzaldehyde	100.0 a	67.0 b	0.0 c	0.0 c	0.0 c	1272.1 a	1532.4 a	0.0 b	0.0 b	0.0 b
Nonanal	76.7 a	67.0 a	15.0 b	0.0 b	0.0 b	925.2 a	754.3 b	519.8 c	0.0 d	0.0 d
(*E*)-2-Heptenal	100.0 a	80.0 a	0.0 b	0.0 b	0.0 b	2741.3 a	1231.2 b	0.0 c	0.0 c	0.0 c
(E)-2-Octenal	76.7 a	60.0 a	0.0 b	0.0 b	0.0 b	897.2 a	1106.8 a	0.0 b	0.0 b	0.0 b
(*E*)-2-Hexenal	73.3 a	66.7 a	0.0 b	0.0 b	0.0 b	91466.0 a	15840.0 b	0.0 b	0.0 b	0.0 b
(E,E)-2,4 Hexadienal	86.7 a	38.2 b	0.0 c	0.0 c	0.0 c	45731.0 a	18271.0 b	0.0 b	0.0 b	0.0 b
Octanal	93.3 a	80.0 b	0.0 c	0.0 c	0.0 c	3656.0 b	15333.0 a	0.0 c	0.0 c	0.0 c
Alcohols										
1-Octen-3-ol	137.0 a	113.0 b	100.0 b	0.0 c	0.0 c	1138.2 a	1253.1 a	1063.2 a	0.0 b	0.0 b
1-Hexanol	90.0 a	120.0 a	106.7 a	23.3 b	0.0 b	22115.0 c	66341.0 b	94101.0 a	8872.0 c	0.0 c
1-Pentanol	90.0 a	93.3 a	40.0 a	103.3 a	50.0 a	95262.0 a	92874.0 a	3597.0 b	151315.0 a	4392.0 b
3-Octanol	76.7 a	70.0 a	30.0 ab	0.0 b	0.0 b	15560.0 a	9628.0 ab	1653.0 b	0.0 b	0.0 b
Ketones										
2-Heptanone	96.2 a	83.5 a	46.6 b	28.7 c	19.0 c	45712.0 b	34218.0 b	121918.0 a	18279.0 b	5085.0 b
3-Hexanone	86.7 a	93.3 a	83.3 a	80.0 a	66.7 a	39177.0 ab	44571.0 a	18433.0 abc	6780.0 bc	527.0 c
3-Heptanone	90.0 a	83.3 a	103.3 a	93.3 a	83.3 a	6376.0 b	6755.0 b	22656.0 a	31600.0 a	6742.0 b
3-Octen-2-one	93.3 a	80.0 a	76.7 a	30.0 b	16.7 b	24381.0 a	4237.0 b	3321.0 b	713.0 b	0.0 b
Furans										
2-Pentyl furan	90.0	83.3	80.0	93.3	90.0	79077.0 ab	32651.0 bc	13918.0 d	93441.0 a	38352.0 bc

Letters represent data significantly ($P < 0.001$) different from other data with different letters.

chambers. Similarly, conidia did not germinate at 50 μL EV in the presence of 1-octen-3-ol, 1-hexanol, and 3-octanol. 1-pentanol was ineffective even at the highest level, 50 μL EV, to prevent *A. flavus* inoculant from germinating and colonizing chambers to a mass comparable to the controls.

Ketones. *A. flavus* colonized all treatment chambers that received a ketone compound regardless of exposure volume, even at the highest dose of 50 μL EV. Colony growth was reduced only by 2-heptanone at 10, 25, and 50 μL EV (68%, 70%, and 80%, respectively), and by 3-octen-2-one at 25 μL EV (68%) and 50 μL EV (82%). *A. flavus* colonization was not affected at any exposure volume by either 3-hexanone or 3-heptanone compared to that of controls.

Furans. Only 1 furan, 2-pentylfuran, was included in the study and did not show a difference in colony growth (dry weight) or AFB1 compared to that of the controls.

Inhibition of AFB1 production

Aldehydes. AFB1 was detected only in agar where fungal colonies formed but AFB1 concentrations varied depending on which volatile was present. AFB1 was detected in all chambers that were exposed to 0 or 1 μL EV. AFB1 was reduced (reduction amount in parenthesis) at 1 μL EV by hexanal (46%), nonanal (19%), (*E*)-2-heptenal (44%), (*E*)-2-hexenal (83%), and 2,4 hexadienal (60%). Despite reducing colony growth at 1 μL EV, octanal stimulated AFB1 production 4-fold compared to the amount produced by controls. Hexanal and nonanal continued to reduce AFB1 production even further (79% and 44%, respectively) at 10 μL EV. The remaining aldehyde-treated cultures grown on agar at 10 μL EV did not produce AFB1. This absence of AFB1 correlated with the absence of any fungal growth in the treated agar plates. All chambers treated with 25 and 50 μL EV of each respective compound did not contain any AFB1, again due to the absence of fungal growth.

Alcohols. Influence among the 5 alcohols tested was inconsistent regarding AFB1 production. AFB1 was detected in the majority of the alcohol-treated chambers regardless of treatment. Some tested alcohols exerted an unexpected stimulatory effect upon AFB1 production. Stimulatory effects were shown by 1-hexanol which showed a stepwise increase in 1 and 10 μL EV treatments (3-fold and 4-fold increase, respectively).

1-pentanol stimulated more AFB1 production at 25 μL EV (1.6× greater than controls) even though a 96% AFB1 decrease was observed in the 10 μL EV treatment with this compound. This alcohol caused a reduction in mycelial mass (dry weight) and secondary mycelial production with a 10 μL EV treatment.

Less AFB1 was produced in the presence of 3-octanol at 10 μL EV. The remaining few inoculated agar plates that were AFB1 free corresponded with those inoculated agar plates that did not show any fungal growth.

Ketones. AFB1 was produced at all EV levels in all chambers with only 1 exception, 3-octen-2-one at 50 μL EV, which was probably due to a lack of fungal growth and little conidiation in the corresponding test chamber. 2-heptanone and 3-heptanone both stimulated AFB1 production at 10 μL EV (270% and 360%, respectively) and 3-heptanone continued this effect to a similar degree at 25 μL EV (496%). Significant reductions in AFB1 occurred in the presence of 3-octen-2-one at each of the EV treatments and at 50 μL EV with 3-hexanone (99%).

Furans. 2-pentylfuran reduced AFB1 at 1, 10, and 50 μL EV (58%, 77%, and 58%, respectively). However, at 25 μL EV AFB1 was produced to a comparable amount of that detected in controls.

A. flavus growth in inoculated kernel assay

Effect of (*E*)-2-hexenal on fungal growth. *A. flavus* growth was visually examined on kernels after 5 d of exposure to the volatile (*E*)-2-hexenal. In the absence of the volatile, primary conidia germinated and produced colonies densely packed with dark green secondary conidia localized at the pedicel end of kernels. Primary conidia also germinated on kernels exposed to 1 μL of the volatile however, colony growth tended to be more floccose with aerial growth and distributed across the kernel length. Mycelia also cascaded over the side of the cap and down onto the bottom of the dish. Secondary conidia were light green with more scattered mycelial growth. Kernels exposed to 10 μL of (*E*)-2-hexenal were not colonized by *A. flavus* . No discoloration was observed on kernels in any of the three treatments. Dry kernel weight did not differ among treatments (Table 2).

(*E*)-2-heptenal effect on fungal growth. Visual examination of *A. flavus* growth on kernels after 5 d of exposure to

Table 2—Effect of (*E*)-2-hexenal and (*E*)-2-heptenal on kernel weight and aflatoxin production by *Aspergillus flavus* on corn kernels.

		(*E*)-2-hexenal			(*E*)-2-heptenal		
		Run			Run		
	Aldehyde volume (μL)	1	2	3	1	2	3
Kernel weight (μg)	0	0.9365	0.8900	0.8467	0.6645	0.6582	0.8667
	1	0.9630	0.8967	0.8467	0.7132	0.6468	0.8700
	10	0.9882	0.8950	0.9050	0.7160	0.6187	0.9617
Aflatoxin B_1 (ng/g corn kernel)	0	0.00	73.20	58.50	14.2	49.7	33.7
	1	34.32	48.50	20.10	24.6	18.7	51.4
	10	0.00	0.00	0.00	0.0	0.1	0.0
Aflatoxin B_1 (ng per petri dish)	0	ND	65.50	49.12	9.70	32.00	30.00
	1	33.26	43.03	16.70	17.60	12.10	44.80
	10	0.00	0.00	0.00	0.00	0.10	0.00
Combined data							
Kernel weight (g)	0	0.89			0.73		
	1	0.90			0.74		
	10	0.93			0.76		
Aflatoxin B_1 (ng/g corn kernel)	0	65.87a			32.62a		
	1	34.31b			31.58a		
	10	0.00c			0.05c		

Letters represent significantly ($P < 0.001$) different data sets ($n = 18$).
ND = not done.

(*E*)-2-heptenal showed a growth pattern similar to that observed with (*E*)-2-hexenal at 1 μL exposure. Colony growth was densely compacted on the pedicel end of kernels and supported a large population of dark green secondary conidia in the control plates. In the presence of 1 μL of (*E*)-2-hexenal, *A. flavus* growth was less dense and randomly scattered compared to that on nontreated kernels. In addition, secondary conidia were dark green and population density varied. Germinated kernels (across the 3 tests, 14% of all control kernels) were observed in several of the control plates and in 1 μL treated chambers. Kernels exposed to 10 μL of (*E*)-2-heptenal were not colonized by *A. flavus*. No discoloration was observed on kernels in any of the 3 treatments. Dry kernel weight did not differ among treatments (Table 2).

AFB1 production

(*E*)-2-hexenal reduction of aflatoxin. AFB1 production was reduced by nearly half in kernels exposed to 1 μL compared to AFB1 recovered from nontreated kernels (Table 2). Kernels exposed to 10 μL of (*E*)-2-hexenal were free of AFB1. Mycelia were not observed on the kernels treated with 10 μL of (*E*)-2-hexenal.

(*E*)-2-heptenal affect on aflatoxin. A comparable concentration of AFB1 was produced in control kernels and kernels exposed to 1 μL EV of (*E*)-2-heptenal (Table 2). At 10 μL EV, however, AFB1 was not detected. Mycelial growth was not present on kernels treated with 10 μL of (*E*)-2-heptenal.

Conclusions

Plants produce many chemicals that either inhibit the growth of, or are lethal to, fungi. Among such compounds are volatiles consisting of naturally occurring aldehydes, acetate esters, alcohols, and terpenes (Hamilton-Kemp and others 1995; Archbold and others 1999; Filonow 2001; Utama and others 2002; Wolken and others 2002; Chitarra and others 2004). Other antimicrobial volatile compounds are produced by the LOX pathway activated after injury to the plant (Hamilton-Kemp and others 1995).

Of these compounds, the aldehydes have been shown to be the most potent antimicrobials (Utama and others 2002). They were not only inhibitory but also lethal to fungal conidia, mycelia, and bacterial cells (Utama and others 2002). Olive oil contains several aldehydes that inhibit the growth of human pathogenic fungi, including *Tricophyton mentagrophytes, Microsporum canis*, and *Candida* spp. (Battinelli and others 2006). Hexanal and octanal, both aldehydes, were also found to be potent inhibitors of AFB1 production (Wright and others 2000). Earlier studies showed that volatile compounds such as alcohols, aldehydes, and ketones inhibited the growth of food decay microorganisms and toxin production.

The present study showed that 18 induced volatiles produced by soybean homogenates in the presence of lipase (Boué and others 2005, unpublished data) of various chemical families differ in their ability to inhibit *A. flavus* growth and AFB1 production. Of these compounds, the induced soybean aldehydes, (*E*)-2-hexenal, and (*E*)-2-heptenal, were among the most potent inhibitors of *A. flavus* toxin production when this fungus was grown on potato dextrose agar and viable corn kernels. Though known liquid volumes of each volatile tested apparently produced the gaseous form of these compounds, the exact concentration of these volatiles in the atmosphere of the petri plate growth chambers was not measured. Therefore, it is not known whether any volatile was lost through leaks in the parafilm. However, volatile odors were detected by smell, so we hypothesize that little, if any volatile, was lost due to leaks in the parafilm seal. Nevertheless, the results indicated that inhibition of *A. flavus* growth and AFB1 production was dependent on volatile concentration.

Many of the effective volatiles studied to date are naturally present in fruits, spices, and herbs and are considered as safe for human consumption (Hamilton-Kemp and others 1995; Utama and others 2002). It is possible that volatiles such as plant-produced aldehydes could supplant synthetic fungicides used to control fungal growth and subsequent toxin formation in postharvest crops.

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